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(54) A Method of chromatographic isolation for non-glyceride components

(57) A method of chromatographic isolation for non-glyceride components (squalenes, carotenes, vitamin E, sterols and/or the like) from a non-glyceride components - comprising compound by , said method including the steps of

a. introducing the non-glyceride components-comprising compound onto a selective adsorbent to allow an adsorption of the non-glyceride components, and subsequently

b. desorbing the non-glyceride components from the

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adsorbent,

wherein
the adsorption and/or desorption of the non-glyceride components is carried out under a supercritical fluid environment.

Description

Field f the Inv nti n

[0001] The invention relates to a method of chromatographic isolation for non-glyceride components particularly squalenes, carotenes, vitamin E (sometimes abbreviated as Vit. E) sterols and/or the like, from crude palm oil, palm oils products and/or by-products, vegetable oils and/or the like non-glyceride components-comprising compounds.

Background of the Invention

[0002] Crude palm oil contains about 5 % of **"non-glyceride components"** which include carotenoids, tocols (tocopherols and tocotrienols), sterols and squalene. The carotenoids, present at 500-700ppm and comprising mainly a and β carotenes, are important constituents with pro-vitamin A activity, possible antitumor formation properties, and other physiological activities. The tocols which are Vitamin E and also natural anti-oxidants, are present at approximately 600 to 1000 ppm in crude palm oil; the major component being the gamma-tocotrienol which has recently been found to have anti-cancer properties besides its known anti-oxidant activity. Tocotrienol has been found to lower blood cholesterol. The sterols consisting mainly of β-sitosterol, stigmasterol and campesterol, provide raw materials for steroid intermediates and drugs. β-Sitosterol also possesses hypocholesterolemic effect. Squalene is an important ingredient for cosmetics. It also shows beneficial physiological properties.

[0003] Several methods have been developed to extract these valuable compounds. In the case of the carotenoids, the known methods can be classified as follows:-

(i) Extraction by saponification e.g. British Patent 567,682; U.S. Patent 2,460,796; U.S. Patent 2,440,029; U.S. Patent 2,572,467; U.S. Patent 2,652,433.

(ii) Iodine method

(iii) Urea process

(iv) Extraction using Fuller's earth or activated carbon, e.g. British Patent 691,924; British Patent 1,562,794; U.S. Patent 2,484,040.

(v) Extraction by selective solvents e.g. U.S. Patent 2,432,021.

(vi) Molecular Distillation.

[0004] In the saponification method (i) the oil is saponified to give soap, glycerol and a non-saponifiable fraction containing carotenes.

[0005] In the iodine method (ii), the iodine is added to a solution of palm oil in petroleum ether, an insoluble precipitate of carotene di-iodide is formed. The iodo-compound when treated with sodium thiosulphate however yields iso-carotene or dehydro-carotene which has no biological activity.

[0006] With the urea method (iii), the triglycerides are broken down to fatty acids and methyl esters which then form insoluble compounds with urea and thiourea, leaving the carotenoids in the remaining liquid.

[0007] Extraction of carotenes using adsorbents has been carried out using Fuller's earth and activated carbon (method iv). However, the extraction of the carotenes from the earth gives oxidised or isomerised products of carotenes. Carotene is concentrated six times in the extract.

[0008] Extraction of carotenes by selective solvents (method v) has been carried out using propane or furfural. The carotene is concentrated (three times that of the original oil) in the furfural phase.

[0009] By method (vi) carotenes can also be obtained by molecular distillation (10^{-3}à- 10^{-4}à mm Hg). Fractions collected at 230°C have a carotene content of about five times that of the original oil.

[0010] To-date, there has not been any method which discloses the use of **supercritical fluid** in the adsorption/desorption chromatography isolation/separation of the non-glyceride components (i.e. carotenoids, tocols, sterols and squalene) from plant source such as CPO (crude palm oil), palm oil products and/or by-products, vegetable oils and fats and/or the like non-glyceride components-comprising compounds.

[0011] All known method previously disclosed in the adsorption/desorption cromatographic separation solely involve the use of solvents (which are costly and hazardous). Thus, there is a need to provide a separation and/or an isolation process, which avoids or discourages the sole use of solvents and consequently rendering it "non-hazardous" for

recovering these non-glyceride components.

Summary of the Invention

[0012] In accordance with the present invention, there is provided a method of chromatographic isolation for non-glyceride components (carotenes, vitamin E [sometimes abbreviated as Vit. E], sterols, squalene) from natural oils and fats, wherein the method comprises the use of supercritical fluid (such as SC-CO_2) in combination with adsorbents (such as silica gel or C18 reverse phase silica gel). The present invention may be subjected directly and/or indirectly to

(a) crude vegetable oil esters (via catalytic alcoholic esterification/transesterification of vegetable oils) or

(b) concentrate (through removal of the bulk of the esters from (a) via vacuum distillation including molecular distillation)

or

(c) the unsaponifiable matters of the concentrate in (b) (through saponification process)

[0013] High concentration of carotenes, vitamin E, sterols and squalene can be obtained by monitoring supercritical fluid process conditions such as temperature between 30°C-100°C, pressure from 50kg/cm^{2}à to 600kg/cm^{2}à , with and without an to entrainer (co-solvent such as alcohols).

[0014] A main advantage of the invention lies in the use of liquified gas at supercritical conditions, hence avoiding or reducing the prior requirements for hazardous solvents.

Detailed description of the Invention

[0015] According to the present invention there is provided a method of chromatographic isolation for "minor non-glyceride components" (including carotenoids, tocols, sterols and squalene) from CPO (crude palm oil), palm oil products and/or by-products, vegetable oil and/.or the like non-glyceride components-comprising compounds, said method comprising the following steps for three different routes as described below:

Route (a)

[0016]

(i) Esterifying the free fatty acid component of the non-glyceride components-comprising compounds with one or more monohydric alcohols to form an esterified compound with a very low free fatty acid content;

(ii) Trans-esterifying the glyceride components with one or more monohydirc alcohols to convert into monoesters;

(iii) Introducing the non-glyceride components-comprising compounds (i.e. esters from steps (i) and (ii) above) to allow the adsorption of non-glyceride components (carotenoids, tocols, sterols and squalene) onto a selective adsorbent; and subsequently

(iv) Desorbing the non-glyceride components from the adsorbent, wherein the adsorption/desorption of the non-glyceride components in steps (iii) - (iv) is carried out under a supercritical fluid environment.

Route (b)

[0017]

(i) Esterifying the free fatty acid components of the non-glyceride components-comprising compounds with one or more monohydric alcohols to form an esterified compound with a very low free fatty acid content;

(ii) Trans-esterifying the glyceride components with one or more monohydirc alcohols to convert into monoesters;

(iii) Removing bulk of the esters in step (i) and (ii) by vacuum distillation including molecular distillation to yield carotene concentrate of 1-8%;

(iv) Introducing the non-glyceride components-comprising compounds (i.e. the carotene concentrate from step (iii) above) to allow the adsorption of non-glyceride components (carotenoids, tocols, sterols and squalene) onto a selective adsorbent; and subsequently

(v) Desorbing the non-glyceride components from the adsorbent, wherein the adsorption/desorption of the non-glyceride components in steps (iv)- (v) is carried out under a supercritical fluid environment.

Route (c)

[0018]

(i) Esterifying the free fatty acid component of the non-glyceride components-comprising compounds with one or more monohydirc alcohols to form an esterifed compound with a very low free fatty acid content;

(ii) Trans-esterifying the glyceride components with one or more monohydric alcohols to convert into monoesters;

(iii) Removing bulk of the esters in step (i) and (ii) by vacuum distillation including molecular distillation to yield carotene concentrate of 1-8%.

(iv) Saponifying the carotene concentrate in step (iii) to yield unsaponifiable matters;

(v) Introducing the non-glyceride components-comprising compounds to allow the adsorption of non-glyceride components (carotenoids, tocols, sterols and squalene) onto a selective adsorbent; and subsequently

(vi) Desorbing the non-glyceride components from the adsorbent, wherein the adsorption/desorption of the non-glyceride components in steps (v)-(vi) is carried out under a supercritical fluid environment.

[0019] Preferably, the esterification of step (i) above is carried out employing

(a) a solid alkali metal bisulphate **or**

(b) a sulphate acid strongly-acidic ion-exchange resin.

[0020] Preferably, the transesterification of step (ii) is carried out employing a basic catalyst .

[0021] Alternatively, both the esterification and transesterification are carried out using an enzyme; for example *candida rugosa* and lipase. 1 to 20% by weight of catalyst may be employed depending upon the weight of the free fatty carboxylic to acid moiety present in the non-glyceride components-comprising compounds.

[0022] Preferably, the monohydric alcohols used in the esterification of step (i) or transesterification of step (ii) comprise one or more C_1 to C_8 alcohols, most preferably methanol.

[0023] Preferably, the selective adsorbent for the adsorption of the non-glyceride components comprise silica gel and/or C18 reverse phase silica gel. The adsorbents may be subjected to adsorption of the non-glyceride components present within anyone of the following;

(i) the esterified palm oil; or

(ii) the carotene concentrate (1-8%) wherein bulk of the esters have been removed through vacuum distillation or molecular distillation (short path distillation); or

(iii) the unsaponifiable materials by saponifying the carotene concentrate of (ii).

[0024] The solvents employed for the present method comprises;

(i) Supercritical fluid, e.g. SC-CO_2(100%), and/or

(ii) Supercritical fluid (e.g. SC-CO_2) in combination with an entrainer (solvent such as alcohol)

[0025] Carotene, tocols, sterols and squalene can be isolated with > 50% purity in a single run by varying pressure from 50 kg/cm^{2}à-600kg/cm^{2}à at temperatures 30°C-100°C.

[0026] It shall therefore be illustrated, by means of the following examples, that supercritical fluid, applied to solid adsorbents such as silica gel and/or C18 reverse phase silica gel, provides a very satisfactory means for isolating non-glyceride components (including carotenoids, tocols, sterols and squalene) from CPO (crude palm oil), palm oil products and/or by-products, vegetable oil and/or the like non-glyceride components-comprising compounds.

Example 1

[0027] Crude palm oil methyl esters containing "minor" non-glyceride components (including carotenes, vitamin E, squalene and sterols), derived from alcoholic esterification/transesterification of crude palm oil with alkali bisulphate or sulphonated ion exchange resin (for esterification) and with 0.3-2% based catalyst and methanol was passed through molecular distillation at a temperature of 90°C-180° and pressure of less than 100mTorr to give a carotene concentrate of 1-8%. The carotene concentrate was subjected to saponification process to obtain unsaponifiable matters containing carotenes, vitamin E, sterols and squalene.

[0028] A typical sample of the unsaponifiable matter of palm carotene concentrate (0.0368g) containing carotenes (21.6%), vitamin E (2.7%), sterols (56.5%) and squalene (8.2%) was dissolved in an organic solvent, such as dichloromethane (2ml) and loaded onto a column (silica gel, with internal diameter 20mm and length: 250mm). The sample mixture was eluted using a mixture of supercritical carbon dioxide (3.Oml, 98.4%) and entrainer, ethanol (0.05ml, 1.6%) with a flowrate of 3.05ml/min. The pressure of the supercritical fluid chromatography system was monitored from P=100kg/cm2 to 300kg/cm2 in 9 hours, keeping temperature constant at 80°C. Various fractions at different time intervals were collected and results are shown in Table 1. Squalene was collected at Pressure 100-180kg/cm^{2}à, Carotene at P=180-220kg/cm^{2}à; Vitamin E at P=220-280kg/cm^{2}à and sterols at >280 kg/cm^{2}à. Total carotenes, vitamin E, sterols and squalene collected in each fraction was determined by UV-visible spectrophotometer at 446nm, HPLC (fluorescent detector, excitation 323nm, and emission 296nm), and GC (for sterols and squalene) respectively.

Example 2

[0029] The procedure of example 1 was repeated except that the palm carotene concentrate (4% concentration) was used instead of unsaponifiable matters of the concentrate to recover carotenes, vitamin E, sterols and squalene separately in different fractions. Results are shown in Table 2.

Example 3

[0030] The procedure of Example 1 was repeated except that crude palm oil methyl esters were used directly to load onto supercritical fluid chromatography to recover carotenes, vitamin E, sterols and squalene separately in different fractions. The results are shown in Table 3.

Example 4

[0031] The procedure of Example 1 using unsaponifiable matters of the carotene concentrate was repeated except that only 100% SC-CO2 (without entrainer) was used for adsorbing/desorbing carotenes, vitamin E, sterols and squalene. Results are shown in Table 4.

Example 5

[0032] The procedure of Example 2 using palm carotene concentrate (4% concentration) was repeated except that only 100% SC-C02 (without entrainer) was used for adsorbing/desorbing carotenes, vitamin E, sterols and squalene. Results are shown in Table 5.

Example 6

[0033] The procedure of Example 3 using crude palm oil methyl esters was repeated except that 100% of SC-C02 (without entrainer) was used. Results are shown in Table 6.

Example 7

[0034] The procedure of Example 1 using unsaponifiable matters of the carotene concentrate was repeated except that the adsorbent used was C18 reverse phase silica gel instead of silica gel. Results are shown in Table 7.

Example 8

[0035] The procedure of Example 2 using palm carotene concentrate (4% concentration) was repeated except that the adsorbent used was C18 reverse phase silica gel instead of silica gel. Results are shown in Table 8.

Example 9

[0036] The procedure of example 3 was repeated except that crude palm oil methyl esters were used directly to load onto supercritical fluid chromatography with C18 reverse phase silica gel to recover carotenes, vitamin E, sterols and squalene. Results are shown in Table 9.

Example 10

[0037] The procedure of example 7 using unsaponifiable matters of the carotene concentrate was repeated except that only 100% SC-C02 (without entrainer) was used for adsorbing/describing carotenes, vitamin E, sterols and squalene onto C18 reverse phase silica gel adsorbent. Results are shown in Table 10.

Example 11

[0038] The procedure of example 8 using palm carotene concentrate (4% concentration) was repeated except that only 100% SC-CO_2 (without entrainer) was used for adsorbing/desorbing carotenes, vitamin E, sterols and squalene onto C18 reverse phase silica gel adsorbent. Results are shown in Table 11.

Example 12

[0039] The procedure of example 9 was repeated except that crude palm oil methyl esters (instead of concentrate), 100% SC-CO_2 (without entrainer) and C_{18} reverse phase silica gel (instead of silica gel) were used. Results are shown in Table 12.

Example 13

[0040] The procedure of Example 1, 2 and 3 were repeated except that other palm oil products such as neutralized palm oil, crude palm olein, crude palm stearin, palm fibre oil and palm fatty acid distillate were used. The results are shown in Table 13. Squalene was collected at pressure 100-180kg/cm^{2}à; carotene at P= 180-220 kg/cm^{2}à; vitamin E at P=220-280 kg/cm^{2}à and sterols at P> 280 kg/cm^{2}à.

Example 14

[0041] The procedure of Example 1, 2 and 3 were repeated except other vegetable oils such as soybean, canola, rapeseed, coconut, sunflower seed, corn oil, olive oil were used. The results are tabulated in Table 14. Squalene was collected at pressure 100-180kg/cm^{2}à; carotene at P= 180-220 kg/cm^{2}à (if any); vitamin E at P=220 280 kg/cm^{2}à and sterols at P> 280 kg/cm^{2}à.

Example 15

[0042] The procedure of Examples 7, 8, and 9 using the same starting materials as described therein were repeated and the results are presented in Table 15. Some variations to the operating conditions are also presented in Table 15 .

Example 16: Isolation of Palm Vitamin E isomers

[0043] Vitamin E concentrate (0.01 g; concentration: -50%) was dissolved in organic solvent, such as dichloromethane or ethanol and loaded onto a column (silica gel, with internal diameter 20mm and length 250mm). The Vitamin E concentrate mixture was eluted using a mixture of supercritical carbon dioxide (5ml, 95.2%) and an entrainer, ethanol (0.25ml, 4.8%) with a flowrate of 5.25ml/min. The pressure of the supercritical fluid chromatography system was kept constant right through the run at P=180kg/cm^{2}à, keeping temperature constant at 70°C. The four vitamin E isomers

were collected at different time interval and are shown in Table 16. The presence and concentration of Vitamin E isomer were confirmed by HPLC as in Example 1.

Example 17: Isolation of Carotenes, Vitamin E, sterols and squalene (Fixed pressure and temperature)

[0044] The procedure of Example 16 was repeated except the unsaponifiable matter of palm carotene concentrate (0.01 g) containing carotenes (21.6%), Vit E (2.7%), sterols (56.5%) and squalene (8.2%) was used. Carotenes, Vit E, sterols and squalene were separated into different fractions and the results are shown in Table 17. The difference between Example 17 and that of Example 1 is that carotenes, Vit E, sterols and squalene can be isolated at fixed temperature (70°C) and pressure (180 kg/cm^{2}à).

Example 18

[0045] The procedures of Examples 16 and 17 were repeated. Unsaponifiable matter of palm caroteneNit E concentrate (0.01g) was used and loaded onto a column (silica gel, with internal diameter 20mm and length 200mm). The results are shown in Table 18. Carotenes, Vit E., sterols and squalene were isolated in different fractions.

Example 19

[0046] The procedures of Examples 16, 17 and 18 were repeated except the temperature: 40°C, pressure: 160kg/cm^{2}à and SC-CO_2: ethanol (v/v=93.8%:6.2%). Unsaponifiable matter of palm carotene/vit. E concentrate (0.005g) was used and loaded onto a column (silica gel, with internal diameter: 4.6mm and length: 250mm). Carotenes, Vit. E, sterols and squalene were isolated in different fractions and results are presented in Table 19.

Table 1:

Unsaponifiable Matters (with entrainer)						
Pressure Fractions	Time	(kg/cm^{2}à)	Squalene (%)	Carotenes (%)	Vitamin E (%)	Sterols (%)
SM	-	-	8.2	21.6	7.7	59.5
F1	0-135	100	86	-	-	-
F2	135-195	180	9.4	83.4	-	-
F3	195-240	220	-	9.2	60.1	-
F4	240-260	250	-	4.4	20.2	-
F5	260-300	250	-	1.5	11.1	
F6	300-360	280	-	0.8	8.8	5.0
F7	360-500	280	-	-	-	95.5

SM: Starting Materials

Table 2:

Carotene Concentrate (with entrainer)						
Fractions	Time (min)	Pressure (kg/cm^{2}à)	Squalene (%)	Carotenes (%)	Vitamin E (%)	Sterols (%)
SM	-	-	9.8	4.1	2.3	5.3
F1	0-135	100	92	-	-	-
F2	135-195	180	4.8	70.6	-	-
F3	195-240	220	-	8.2	13.8	-
F4	240-260	250	-	3.1	57.3	-
F5	260-300	250	-	3.2	22.5	-
F6	300-360	280	-	1.4	6.6	2.4
F7	360-500	280	-	-	-	96.6

SM: Starting Materials

Table 3:

Crude Palm Oil Methyl Est rs (with entrainer)						
Fractions	Time (min)	Pressure (kg/cm^{2}à)	Squalene (%)	Carotenes (%)	Vitamin E (%)	Sterols (%)
SM	-	-	0.03	0.06	0.06	0.04
F1	0-135	100	3.6	-	-	-
F2	135-195	180	-	10.2	-	-
F3	195-240	220	-	3.2	1.2	-
F4	240-260	250	-	1.2	12.6	-
F5	260-300	250	-	12.0	10.8	-
F6	300-360	280	-	-	2.8	0.8
F7	360-500	280	-	-	-	3.6

SM: Starting Materials

Table 4:

Unsaponifiable matters (without entrainer)						
Fractions	Time (min)	Pressure (kg/cm^{2}à)	Squalene (%)	Carotenes (%)	Vitamin E (%)	Sterols (%)
SM	-	-	8.2	21.6	7.7	59.5
F1	0-100	100	36	-	-	-
F2	100-160	180	88	2	-	-
F3	160-200	220	-	81.3	0.8	-
F4	200-240	250	-	11.7	12.7	-
F5	240-260	250	-	6.6	73.6	0.9
F6	260-300	300	-	-	8.8	8.2
F7	300-450	300	-	-	-	90.2

SM: Starting Materials

Table 5:

Carotenes Concentrate (without entrainer)						
Fractions	Time (min)	Pressure (kg/cm^{2}à)	Squalene (%)	Carotenes (%)	Vitamin E (%)	Sterols (%)
SM	-	-	9.8	4.1	2.3	5.3
F1	0-135	100	24	-	-	-
F2	135-195	180	76	-	-	-
F3	195-240	220	-	67.8	18.7	-
F4	240-260	250	-	18.9	58.9	-
F5	260-300	250	-	8.7	20.3	-
F6	300-360	280	-	0.2	6.1	11.4
F7	360-500	280	-	-	-	83.3

SM: Starting Materials

Table 6:

Crude Palm Oil M thyl Esters (without entrainer)						
Fractions	Time (min)	Pressure (kg/cm^{2}à)	Squalene (%)	Carotenes (%)	Vitamin E (%)	Sterols (%)
SM	-	-	0.03	0.06	0.06	0.04
F1	0-135	100	-	-	-	-
F2	135-195	180	8.6	-	-	-
F3	195-240	220	-	10.8	-	-
F4	240-260	250	-	8.7	20.8	-
F5	260-300	250	-	6.8	16.7	-
F6	300-360	280	-	-	6.6	1.5
F7	360-500	280	-	-	-	8.6

SM: Starting Materials

Table 7: Unsaponifiable matter (with entrainer)

Fractions	Time (min)	Pressure (kg/cm^2)	Compounds (%)			
			Squalene	Vitamin E	Carotenes	Sterols
Starting materials	—	—	6.8	9.8	20.8	42.8
F1	0 – 70	0 – 10min — 100 kg/cm^2 10 – 20min — 140 kg/cm^2	83.8	—	—	—
F2	70 – 100	20 – 55min — 160 kg/cm^2 55 – 65min — 180 kg/cm^2 65 – 90min — 200 kg/cm^2	12.3	8.3	—	—
F3	100 – 140	90 – 300min — 250 kg/cm^2	—	16	0.3	—
F4	140 – 170	> 250 kg/cm^2	—	1.8	8.6	—
F5	170 – 200	> 250 kg/cm^2	—	0.5	22.8	—
F6	200 – 250	> 250 kg/cm^2	—	—	99.4	10.2
F7	250 – 450	> 250 kg/cm^2	—	—	—	88.1

Temperature: 80°C
Flowrate: 6.0/0.2 ml/min (SC-CO_2 : ethanol)

Table 8: Carotene Concentrate (with entrainer : ethanol)

Fractions	Time (min)	Pressure (kg/cm^2)	Compounds (%)			
			Squalene	Vitamin E	Carotenes	Sterols
Starting materials	—	—	0.8	2.15	8.06	9.22
F1	0 – 70	0 – 10min — 100 kg/cm^2 10 – 20min — 140 kg/cm^2	66.8	—	—	—
F2	70 – 100	20 – 55min — 160 kg/cm^2 55 – 65min — 180 kg/cm^2 65 – 90min — 200 kg/cm^2	7.5	76	0.2	—
F3	100 – 140	90 – 300min — 250 kg/cm^2	—	18	0.2	—
F4	140 – 170	> 250 kg/cm^2	—	2	9.3	—
F5	170 – 200	> 250 kg/cm^2	—	0.6	32.2	—
F6	200 – 250	> 250 kg/cm^2	—	—	98.1	11
F7	250 – 450	> 250 kg/cm^2	—	—	—	83

Temperature: 80°C
Flowrate: 6.0/0.2 ml/min (SC-CO_2 : ethanol)

Table 9: Crude Palm Oil Methyl Esters (with entrainer)

Fractions	Time (min)	Pressure (kg/cm^2)	Compounds (%)			
			Squalene	Vitamin E	Carotenes	Sterols
Starting materials	—	—	0.03	0.06	0.06	0.04
F1	0 – 70	0 – 10min — 100 kg/cm^2 10 – 20min — 140 kg/cm^2	44.2	—	—	—
F2	70 – 100	20 – 55min — 160 kg/cm^2 55 – 65min — 180 kg/cm^2 65 – 90min — 200 kg/cm^2	3.3	58.2	—	—
F3	100 – 140	90 – 300min — 250 kg/cm^2	—	16.6	0.1	—
F4	140 – 170	> 250 kg/cm^2	—	4.8	—	—
F5	170 – 200	> 250 kg/cm^2	—	1.0	16.8	—
F6	200 – 250	> 250 kg/cm^2	—	—	46.6	5.7
F7	250 – 450	> 250 kg/cm^2	—	—	—	57.8

Temperature: 80°C
Flowrate: 6.0/0.2 ml/min (SC-CO_2 : ethanol)

Table 10: Unsaponifiable matter (without entrainer)

Fractions	Time (min)	Pressure (kg/cm^2)	Compounds (%)			
			Squalene	Vitamin E	Carotenes	Sterols
Starting materials	—	—	6.8	9.8	20.8	42.8
F1	0 – 50	0 – 10min — 100 kg/cm^2 10 – 20min — 140 kg/cm^2	60	—	—	—
F2	50 – 70	20 – 55min — 160 kg/cm^2 55 – 100min — 180 kg/cm^2 100 – 140min — 200 kg/cm^2	89	7.6	0.01	—
F3	70 – 90	140 – 180min — 220 kg/cm^2 180 – 280min — 240 kg/cm^2 280 – 300min — 300 kg/cm^2	—	83	0.04	—
F4	90 – 110	300 kg/cm^2	—	59	0.02	—
F5	110 – 130	300 kg/cm^2	—	52	0.02	—
F6	130 – 145	300 kg/cm^2	—	4	0.2	2.3
F7	145 – 170	300 kg/cm^2	—	8	0.4	6.8
F8	170 – 300	300 kg/cm^2	—	—	99.1	8.6
F9	300 – 450	300 kg/cm^2	—	—	—	80.3

Temperature: 80°C
Flowrate: 6.0 ml/min

Table 11: Carotene Concentrate (without entrainer)

Fractions	Time (min)	Pressure (kg/cm^2)	Compounds (%)			
			Squalene	Vitamin E	Carotenes	Sterols
Starting materials	—	—	0.8	2.15	8.06	9.22
F1	0 – 50	0 – 10min — 100 kg/cm^2 10 – 20min — 140 kg/cm^2	53	—	—	—
F2	50 – 70	20 – 55min — 160 kg/cm^2 55 – 100min — 180 kg/cm^2 100 – 140min — 200 kg/cm^2	81	9.8	0.03	—
F3	70 – 90	140 – 180min — 220 kg/cm^2 180 – 280min — 240 kg/cm^2 280 – 300min — 300 kg/cm^2	—	72	0.2	—
F4	90 – 110	300 kg/cm^2	—	59	0.08	—
F5	110 – 130	300 kg/cm^2	—	51	0.03	—
F6	130 – 145	300 kg/cm^2	—	6	0.8	8.4
F7	145 – 170	300 kg/cm^2	—	10	1.7	7.6
F8	170 – 300	300 kg/cm^2	—	—	98.3	2.7
F9	300 – 450	300 kg/cm^2		—	—	70.3

Temperature: 80°C
Flowrate: 6.0 ml/min

Table 12: Crude Palm Oil Methyl Esters (without entrainer)

Fractions	Time (min)	Pressure (kg/cm^2)	Compounds (%)			
			Squalene	Vitamin E	Carotenes	Sterols
Starting materials	—	—	0.03	0.06	0.06	0.04
F1	0 – 50	0 – 10min — 100 kg/cm^2 10 – 20min — 140 kg/cm^2	20	—	—	—
F2	50 – 70	20 – 55min — 160 kg/cm^2 55 – 100min — 180 kg/cm^2 100 – 140min — 200 kg/cm^2	61	—	—	—
F3	70 – 90	140 – 180min — 220 kg/cm^2 180 – 280min — 240 kg/cm^2 280 – 300min — 300 kg/cm^2	—	56.7	—	—
F4	90 – 110	300 kg/cm^2	—	32.2	0.8	—
F5	110 – 130	300 kg/cm^2	—	31.2	1.6	—
F6	130 – 145	300 kg/cm^2	—	1.3	1.8	—
F7	145 – 170	300 kg/cm^2	—	—	12.2	3.2
F8	170 – 300	300 kg/cm^2	—	—	57.8	4.6
F9	300 – 450	300 kg/cm^2	—	—	—	58.3

Temperature: 80°C
Flowrate: 6.0 ml/min

Table 13

(Palm oil products and bv-products)					
Palm Oil Products		Compounds (%)			
		Squalene	Carotene	Vitamin E	Sterols
1.	CPOs	0.012	0.032	0.042	0.026
	ME CPOs	28.7	24.4	18.2	26.7
	Concentrate CPOs	56.2	48.8	44.3	56.8
	Unsap. matters CPOs	76.8	63.3	60.5	80.1
2.	CPOo	0.022	0.092	0.076	0.033
	ME CPOo	30.6	38.6	36.4	44.5
	Concentrate CPOo	63.1	78.2	64.3	79.9
	Unsap. matters CPOo	85.7	95.2	89.1	98.1
3.	NPO	0.034	0.065	0.066	0.044
	ME NPO	32.1	40.4	41.1	55.4
	Concentrate NPO	65.0	62.5	60.6	88.2
	Unsap. matters NPO	88.1	81.8	80.7	96.9
4.	PFAD	0.8	—	0.78	1.12
	ME PFAD	36.7	—	45.5	50.8
	Concentrate PFAD	73.3	—	79.4	80.6
	Unsap. matters PFAD	92.6	—	88.7	91.3
5.	Residual fibre oil	0.056	0.52	0.23	0.48
	RME fibre oil	30.2	38.9	38.8	57.7
	Concentrate R fibre oil	76.3	60.1	58.6	78.8
	Unsap. matters R fibre oil	90.7	88.3	82.1	93.3

CPOs crude palm stearin
CPOo crude palm olein
NPO neutralised palm oil
PFAD palm fatty acid distillate
R residual
ME methyl esters

Table 14:

Vegetable oils				
Vegetable Oils		Compounds (%)		
		Squalene	Vitamin E	Sterols
1.	Soyabean Oil (SO)		0.116	0.320
	ME SO	—	27.7	32.2
	Concentrate SO		52.9	60.7
	Unsap. matter SO		72.8	90.1
2.	Sunflower seed (SU)		0.064	0.362
	ME SU	—	20.1	33.3
	Concentrate SU		50.3	61.8
	Unsap. matter SU		63.9	91.5
3.	Rapeseed oil (Ra)		0.027	0.582
	ME Ra	—	21.0	35.7
	Concentrate Ra		43.7	65.4
	Unsap. matter Ra		58.5	89.5

Veg table oils				
Vegetable Oils		Compounds (%)		
		Squalene	Vitamin E	Sterols
4.	Rice bran (Ri) .		0.098	1.52
	ME Ri	—	21.6	46.3
	Concentrate Ri		48.2	68.2
	Unsap. matter Ri		65.8	96.1
5.	Olive oil (OL)	0.688	0.01	0.11
	ME OL	26.3	16.6	32.1
	Concentrate OL	62.0	38.8	60.6
	Unsap. matter OL	89.6	58.1	88.6
6.	Corn Oil		0.060	1.02
	ME corn oil	—	21.2	44.4
	Concentrate corn oil		41.8	65.8
	Unsap. matter corn oil		60.5	92.0
7.	Coconut oil			0.10
	ME coconut oil	—	—	31.3
	Concentrate coconut oil			58.5
	Unsap. matter coconut oil			84.7

Table 15

Fractions	Collctn time (min)	Eluent CO_2/ent. ml/min	Cpds %	(A) Unsap. matters m	(B) Carotene Conc.	(C) Crude Palm Oil Methyl Esters
1	0-90	6ml/0ml	Methyl esters	-	20	99.9
			Squalene	95	80	0.02
2	90-190	6ml/0ml &	Vit. E	81	76.3	68.9
		6ml/0.2ml	Sterols	18.9	14.2	6.2%
3	190-210	6ml/0.2ml &	Sterols	80.2	54.2	26.1
		6ml/0.4ml	Vit. E	18.8	16.8	10.9
4	210-270	6ml/1ml	Carotenes	96	82.3	54.7
Operating pressure: 180 kg/cm^2à						

Table 16:

Isolation of Palm Vit. E isomers (α-T, α-T3, γ-T3 and δ-T3)					
Fractions	Collection Time (min)	Vit. E α-T	Vit. E α-T3	Vit. E γ-T3	Vit. E δ-T3
S/M	-	12.7	11.4	19	5.8
1					
2	41-47	100			
3	47-54		100		
4	54-61			100	

Isolation of Palm Vit. E is mers (α-T, α-T3, γ-T3 and δ-T3)					
Fractions	Collection Time (min)	Vit. E α-T	Vit. E α-T3	Vit. E γ-T3	Vit. E δ-T3
5	61-77			99	1
6	77-145			33	97
7	145-				

a. S/M: Starting material (Vit E concentrate@50%)

b. T: tocopherol

c. T3: tocotrienol

d. Conditions: P= 180kg/cm^{2}à, T= 70°C

e. SC-CO_2: ethanol = 5ml:0.25ml (95.2%:4.8%)

Table 17:

Unsaponifiable matters (with entrainer)					
Fractions	Time (min)	Squalene (%)	Carotenes (%)	Vit. E (%)	Sterols (%)
S/M	-	8.2	21.6	7.7	59.5
F1	0-34	98.0			
F2	34-41		90.0		
F3	41-47		20.0	(α-T) 80.0	
F4	47-54			(α-T3) 100.0	
F5	54-61			(α-T3) 100.0	
F6	61-72			(γ-T3) 100.0	
F7	72-77			(γ-T3) 10.0	90.0
F8	77-145			(δ-T3) 100.0	

Conditions: P= 180kg/cm^{2}à, T=70°C

SC-CO_2: ethanol = 5ml: 0.25ml (95.2%:4.8%, v/v)

Table 18:

Carotene and Vit. E Concentrate					
Fractions	Time (min)	Squalene (%)	Carotenes (%)	Vit. E (%)	Sterols (%)
S/M		8.2	21.6	48.8	21.3
F1	0-7	96			
F2	7-11		90	(α-T) 10.0	
F3	11-13		15	(α-T) 85.0	
F4	13-14			(α-T3) 98.0	
F5	15-20			(γ-T3) 98.0	
F6	20-25			(δ-T3) 98.0	
F7	25-40				98

Silica column: 20mm ID x 200 mm length

Condition: P = 180 kg/cm^{2}à, T = 70°C

SC-CO_2: ethanol = (95%:5%, v/v)

Table 19

Fractions	Time (min)	Squalene (%)	Carotenes (%)	Vit. E (%)	Sterols (%)
S/M		8.2	21.6	48.8	21.3
1	0-1.5	98			
2	1.5-2.5		92		
3	2.5-3.2			(α-T) 96.0	
4	3.2-3.8			(α-T3) 98.0	
5	3.8-4.2			(γ-T3) 98.0	
6	4.2-5			(δ-T3) 98.0	
7	5-8				99

Conditions: P= 160 kg/cm^{2}à, T=40°C

SC-CO_2: ethanol = 3.0:0.2 (mls/min) (93.75%:6.25%, v/v)

Claims

1. A method of chromatographic isolation of non-glyceride components (squalenes, carotenes, vitamin E, sterols and/or the like) from a non-glyceride components-comprising compound by, said method including the steps of

   a. introducing the non-glyceride components-comprising compound onto a selective adsorbent to allow an adsorption of the non-glyceride components, and subsequently

   b. desorbing the non-glyceride components from the adsorbent,
   wherein the adsorption and/or desorption of the non-glyceride components is carried out under a supercritical fluid environment.

2. A method for chromatographic isolation according to claim 1, wherein the selective adsorbents includes silica gel, reverse phase C18 silica gel and/or the like.

3. A method of chromatographic isolation according to claim 1, wherein the adsorption/desorption of the non-glyceride components are carried out under a supercritical fluid environment comprising an effective amount of CO_2 as a solvent.

4. A method of chromatographic isolation according to claim 1, wherein the adsorption/desorption of the non-glyceride components are carried out under a supercritical fluid environment comprising an effective amount of, a combination of C02 and an entrainer, as a solvent.

5. A method of chromatographic isolation according to claim 4, wherein the entrainer comprises an organic solvent.

6. A method of chromatographic isolation according to claim 5, wherein the organic solvent comprises an alky alcohol.

7. A method of chromatographic isolation according to claims 3-6, wherein the supercritical fluid conditions comprise operating temperatures at T= 30°C-100°C and P = 50kg/cm^{2}à- 600kg/cm^{2}à.

8. A method of chromatographic isolation according to claim 1, said method comprising the prior additional steps of

(i) esterifying the free fatty acid components of the non-glyceride components comprising compounds with one or more monohydric alcohols to form an esterified non-glyceride components-containing compounds comprising a very low free fatty acid content;

(ii) trans-esterifying the glyceride components into monoesters by employing one or more monohydric alcohols; wherein the non-glyceride components-comprising compounds, introduced onto the selective adsorbent according to claim 1, contain or comprise the esters from steps (i) and (ii) above.

9. A method of chromatographic isolation according to claim 8, wherein

step (i) is carried out employing a solid alkali metal bisulfate and/or a sulfate acid strongly acidic ion-exchange resin as a catalyst;

step (ii) is carried out employing basic catalyst;

or wherein
both the esterification and trans-esterification of both steps (i)- (ii) are carried out using an enzyme.

10. A method of chromatographic isolation according to claim 8, wherein the esterification and/or trans-esterification of steps (i) - (ii) comprises the use of one or more C1 to C8 alcohols.

11. A method of chromatographic isolation according to claim 10, wherein the esterification and/or trans-esterification comprises the use of methanol.

12. A method of chromatographic isolation accoding to claim 8, wherein the selective adsorbent comprises silica gel and/or reverse-phase C18 silica gel.

13. A method of chromatographic isolation according to claim 8, wherein the adsorption/desorption of the non-glyceride components subsequent to the esterification and/or trans-esterification steps are carried out under a supercritical fluid environment comprising an effective amount of C02 as a solvent.

14. A method of chromatographic isolation according to claim 8, wherein the adsorption/desorption of the non-glyceride components subsequent to the esterification and/or trans-esterification steps are carried out under a supercritical fluid environment comprising an effective amount of, a combination of C02 and an entrainer, as a solvent.

15. A method of chromatographic isolation according to claim 15, wherein the entrainer comprises an organic solvent.

16. A method of chromatographic isolation according to claim 14, wherein the organic solvent comprises an alkyl alcohol.

17. A method of chromatographic isolation according to claims 13-16, wherein the supercritical fluid conditions comprise operating temperatures of T= 30°C -100°C and P = 50kg/cm^{2}à- 600kg/cm^{2}à.

18. A method of chromatographic isolation according to claim 8, said method comprising the additional step of removing bulk of the esters by vacuum distillation including molecular distillation to yield carotene concentrate of 1-8%, wherein this additional step is taken subsequent to the esterification and trans-esterification steps and prior to the introduction of the non-glyceride components-comprising compounds onto the selective adsorbent, and wherein the non-glyceride components-comprising compounds introduced onto the selective adsorbent contain or comprise the carotene concentrate from the distillation step.

19. A method of chromatographic isolation according to claim 8, said method comprising the additional step of saponifying the carotene concentrate to yield unsaponifiable matters, wherein this additional step is taken subsequent to the esterification and trans-esterification steps and the distillation step, and prior to the introduction of the non-glyceride components-comprising compounds onto the selective adsorbent, and wherein the

non-glyceride components-comprising compounds introduced onto the selective adsorbent contain or comprise the unsaponifiable matters.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 30 9745

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14 February 2001	Rooney, K

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Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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